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Up a Notch: Instructing Gliogenesis

Minireview

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How Do Multipotent Neural Stem Cells Generate Glia?

Our brains contain two principle classes of cells, neurons and glia, which are structurally and functionally distinct. This simple observation raises challenging questions of fundamental importance to neurobiologists. How are neurons and glia generated, and how do they cooperate to control brain function? These questions need to be solved if we are ever to rebuild injured brains and successfully restore function.

How do multipotent neural stem cells decide whether to generate neurons or glial cells? In culture, neural stem cells differentiate into neurons in response to instructive extracellular signals that promote the production or activity of proneural transcription factors. Similarly, neural stem cells differentiate into glia in response to a variety of extracellular signals such as ciliary neurotrophic factor (CNTF), bone morphogenetic proteins (BMPs), transforming growth factor (TGFα), and neuregulin-1 (Nrg1)/glial growth factor-2 (GGF2). But it is not known whether these signals normally induce glial differentiation in vivo or how they act.

Whereas most of these glial-inducing signals are thought to be secreted proteins that act at long range, three groups have just identified a contact-mediated signaling system that is the strongest inducer of glial differentiation to be identified to date. Remarkably, the new glial-inducing signal is Notch activation, best known to neurobiologists for its ability to strongly inhibit the differentiation of neural stem cells. Notch signaling is mediated by a highly conserved family of homologous transmembrane proteins and ligands that control cell fate through local interactions. Though it makes sense that Notch activation might indirectly play an important role in preserving a pool of stem cells for gliogenesis, the ability of Notch signaling to directly stimulate gliogenesis comes as a total surprise. Previous studies had found that Notch signaling strongly inhibits neurogenesis and oligodendrocyte generation, but there had been no sign of any strong effects of Notch on astrocyte differentiation (Nye et al., 1994; Wang et al., 1998). Nonetheless, Notch activation has now been found to trigger the differentiation of three different types of glial cells, including Schwann cells (Morrison et al., 2000), radial glia (Gaiano et al., 2000), and Müller cells (Furukawa et al., 2000). The purpose of this review is to summarize these new studies and to discuss their possible significance.

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Induction of Peripheral Gliogenesis by Notch Activation In Vitro

The peripheral nervous system (PNS) is an ideal location to study gliogenesis, as Schwann cells are the main glial cell type. Neural crest stem cells (NCSCs), the self-renewing multipotent cells that give rise to many PNS neurons and glia, can be purified and generate neurons, glia, and myofibroblasts in vitro in response to known instructive signals (Morrison et al., 1999). For instance, BMPs promote neuronal differentiation, whereas Nrg1/GGF2 promotes Schwann cell differentiation (Shah et al., 1994).

To examine the possible effects of Notch signaling on their differentiation, Morrison et al. (2000) exposed purified NCSCs in culture to a soluble form of a Notch ligand. As expected, Notch activation strongly inhibited neuronal differentiation and myogenesis but, contrary to expectation, enormously enhanced Schwann cell generation. Clonal analysis revealed that the enhanced Schwann cell generation was caused by promotion of differentiation rather than by promoting stem cell self-renewal, maintenance of the undifferentiated state, or survival. Compared to Nrg1, Notch activation significantly enhanced both the amount and the rate of glial differentiation. These studies provide evidence that Notch activation instructively promotes glial differentiation.

Remarkably, unlike Nrg1/GG2, Notch signaling was sufficient on its own to override the strong instructive effects of BMP2 in inducing neuronal differentiation. A short period of Notch activation for only 1 day produced four times more Schwann cell differentiation at the expense of neurogenesis during a 4-day treatment period with BMP2. Thus, Notch activation triggers an irreversible, cell-heritable switch in NCSCs to gliogenesis.

Notch Signaling Promotes Retinal Müller Glia and Cortical Radial Glia Development In Vivo

Whereas the studies of Morrison et al. (2000) focused on effects of Notch activation on PNS cells in vitro, Notch activation has also been found to promote gliogenesis within the developing CNS in vivo. The vertebrate retina provides an ideal location to address this issue, as both retinal neurons and Müller glia are derived from a common stem cell (Turner and Cepko, 1987). Retinal cells are generated in a characteristic order with Müller glia, rod photoreceptor, and bipolar interneurons being born last. As in other brain regions, it is still unclear what signals induce retinal stem cells to generate Müller glia, though TGFα has been proposed to play an instructive role. To examine whether Notch signaling regulated retinal gliogenesis, Furukawa et al. (2000) used retroviral vectors to infect retinal stem cells with a constitutively active form of the Notch receptor. Greater than 90% of progenitor cells infected with this retrovirus generated Müller glial cells, compared to only 8% of cells infected with a control virus. This increase in glial generation came at the expense of other retinal cell types, as the numbers of bipolar cells and rods generated were both significantly decreased. These findings demonstrate that Notch activation specifically promotes stem cells

to generate glia rather than simply inhibiting their differentiation and preserving them as stem cells, as might have been expected. The simplest possibility is that Notch acted directly on retinal stem cells to instructively promote glial differentiation, but other mechanisms remain possible. An increase in glia might be attributed to a prolonged glial proliferative period, for example.

Notch signaling also promotes radial glial differentiation in the murine forebrain. Though for the most part neurogenesis precedes gliogenesis, it has long been recognized that radial glial cells appear concurrently with the the onset of cortical neurogenesis. Radial glia serves as the scaffold along which newborn neurons migrate from the ventricular zone to the developing cerebral cortex. To study whether Notch plays any role in radial glial formation, Gaiano et al. (2000) also used retroviral vectors encoding an active form of Notch receptor. Using a novel ultrasound-based guidance system, they injected concentrated control and constitutively active Notch-encoding retroviruses into the telecenphalic vesicles of embryonic day 9.5 mouse embryo, and injected embryos were sectioned and stained several days or weeks later. Control cells were found distributed throughout the brain, whereas Notch-infected cells were typically found in clusters along the ventricular surface. Most Notch-infected cells resembled radial glia, possessing long processes that extended toward the pial surface and expressing characteristic radial glial markers such as RC2 and brain lipid binding protein (BLBP), whereas few of the control cells had a radial glial phenotype. Thus, Notch activation enormously enhanced the generation of radial glial cells by cortical stem cells. In addition, as expected, Notch decreased the generation of oligodendrocytes and neurons.

Is Notch Signaling Acting Instructively to Promote Gliogenesis?

These three new studies together provide compelling evidence that Notch signaling enhances the formation of a variety of types of glial cells in the CNS and PNS, including radial glia, Müller glia, and Schwann cells, and raise the question of how Notch signaling stimulates gliogenesis. Notch signaling has recently been shown to promote the differentiation of T cell subtypes, epidermis, and myeloid cells, but as for glia, the intracellular molecular mechanism by which it promotes differentiation is unclear.

The simplest possibility is that Notch signaling acts instructively to induce glial differentiation. Notch signaling inhibits neurogenesis by activating the transcription factor CBF1/RBP-J, a mammalian homolog of *Drosophila* Suppressor of Hairless, Su(H), which enters the nucleus and upregulates Hes basic-helix-loop-helix (bHLH) transcription factors, such as Hes1 and Hes5, that bind to and inhibit the proneural gene Mash1 (Kageyama and Ohtsuka, 1999). Conceivably, Notch signaling, via either CBF1/RBP-J or Hes1/5 proteins, might upregulate other transcription factors that induce gliogenesis. In fact, overexpression of either Hes1 or Hes5 is sufficient to enhance Müller glial differentiation (Furukawa et al., 2000; Hojo et al., 2000). Hes proteins in turn might upregulate transcription factors that direct glial differentiation. However, transcription factors that are sufficient to induce vertebrate glial differentiation within the nervous system have not yet been identified. Alternatively, gliogenesis might be a default fate that can be expressed only if Mash1, or related proneural proteins, are not present or are inhibited by Hes or other proteins.

Is it also possible that Notch signaling promotes gliogenesis by maintaining or enhancing responsiveness of multipotent stem cells to instructive signals? This possibility should not be easily dismissed, since many known extracellular signaling molecules can instructively induce gliogenesis in vitro. Notch signaling has already been shown to regulate the competence of certain cells to respond to LIN-3, an EGF family homolog (Wang and Sternberg, 1999). Interestingly, both of the instructive signals previously implicated in Müller glia, radial glia, and Schwann cell differentiation, TGFα and NRG1/GGF2, are also EGF family homologs. Although the new studies on Notch-enhanced Müller and radial glia development in vivo were not accompanied by mechanistic analysis of how Notch acts on precursor cells in vitro, the clonal analyses of Morrison et al. (2000) demonstrate unequivocally that Notch enhances glial generation by acting directly on stem cells. But this does not exclude the possibility that Notch acted by prolonging or enabling responsiveness to an instructive signal that was in fact present in the culture medium.

An important limitation of all of the new studies is that there is not yet any evidence that Notch signaling helps to promote gliogenesis during normal mammalian development. Transgenic mice deficient in Notch signaling, which inevitably die early before gliogenesis begins, cannot be used to address this question. Müller glia production, however, is significantly decreased in transgenic mice lacking Hes5 (Hojo et al., 2000) and by dominant-negative blockade of Hes1 (Furukawa et al., 2000). Thus, it is possible that Notch signaling may normally help to promote the differentiation of at least some types of glial cells.

What Would Be The Point of Using Notch Activation to Signal Gliogenesis?

Multipotent stem cells need to generate many different cell fates and to pattern them appropriately. It is unlikely that a stem cell could make sense of a sea of soluble signals alone to successfully build a brain. A contact-mediated signal, such as Notch, that could help to regulate competency to respond to instructive signals, is a tremendously attractive mechanism for controlling cell patterning and fate simultaneously (Wang and Sternberg, 1999). Contact-dependent signals that control patterning in development have received much attention in simple invertebrate systems, but their role in vertebrate brain development deserves much more attention. This is shown by the studies of Tsai and McKay (2000), who recently found that cell contact does in fact help to regulate the fate choice of cortical neural stem cells, strongly favoring astrocyte generation. Although these investigators did not identify the contact-mediated signal, Notch signaling is obviously an intriguing possibility.

Notch signaling is well documented to mediate lateral inhibitory cellular interactions that partition fates among cells within equivalence groups, helping to achieve appropriate patterning concurrently with cellular diversity (Artavanis-Tsakonas et al., 1999). For instance, in the inner ear, a lateral inhibitory Notch signaling mechanism coordinates the development of the mosaic of sensory

receptor hair cells and glia-like supporting cells (Lanford et al., 1999). A similar Notch-mediated mechanism could conceivably divide an initially equivalent pool of neural stem cells in the ventricular zone into committed neuroblasts and glioblasts. Such a model has long been proposed by neurohistologists (Barres, 1999). In accord with this possibility, Gaiano et al. (2000) propose that early in the development of the cerebral cortex, newly formed neurons expressing Notch ligands activate Notch receptors on nearby neural stem cells within the ventricular zone, thereby inducing them to become radial glia. This would not only provide a nearby radial migratory pathway for the newly formed neurons but, if Notch acts to trigger an irreversible and heritable switch to gliogenesis as it does for Schwann cells, would also preserve a pool of radial glial cells as committed glioblasts. Such a model would help to explain why astrocytes are generated after neurons in the CNS, particularly if radial glial cells are a major quantitative source of astrocytes in the brain. And, if Notch promotes the generation of radial glia and not the astrocytes they generate, this model would also help to explain why effects of Notch on astrocyte generation have not been observed previously.

The new finding that Notch activation helps to regulate which ventricular stem cells become radial glia raises a puzzling question. How can Notch activation help to control which stem cells become radial glial cells when Notch is already thought to control whether stem cells become neurons? One possibility is that Notch has different actions at different times of development—for instance, first partitioning stem cells into committed neuroblasts and glioblasts and later helping to regulate whether neuroblasts differentiate into neurons or self-renew. Another possibility is that Notch has different effects on different lineage stages. For instance, Notch may induce stem cells to become glia but inhibit the differentiation of committed neuroblasts. Finally, the qualitative outcome of Notch signaling might depend on the presence of yet to be identified extracellular signals.

What Is the Relationship between Radial Glia, Stem Cells, and Astrocytes?

The studies of Gaiano et al. (2000) provide new insight into the possible generative functions of radial glial cells. In addition to their well-known function as guide cells for migratory nascent neurons, it has been proposed that radial glial cells are multipotent neural stem cells or glioblasts committed to generating astrocytes. Although radial glia do not divide during neurogenesis and neuronal migration, they begin to divide during gliogenesis. After neurogenesis has ended, radial glia in the cerebral cortex disappear, and, based on histological studies, some of them transform into astrocytes and ependymal cells (Schmechel and Rakic, 1979). The alkaline phosphatase reporter gene used to visualize cells infected by the retroviral vectors used in the experiments of Gaiano et al. (2000) offered an excellent opportunity to further investigate the postnatal transformation of radial glia. Gaiano et al. (2000) confirmed that postnatally many of the radial glial cells gave rise to astrocytes dispersed throughout the brain, providing direct evidence that radial glia are glioblasts that are capable of generating astrocytes.

Gaiano et al. (2000) also found, however, that radial glia gave rise to many subventricular zone astrocytes and ependymal cells. This is quite provocative, as recent studies have shown that a subset of subventricular zone astrocytes and ependymal cells are multipotent neural stem cells in the adult brain (Barres, 1999). This suggests that some radial glia might conceivably be multipotent neural stem cells, a possibility consistent with previous retroviral lineage studies (Gray and Sanes, 1992). There is not yet compelling evidence, however, that any of the SVZ astrocytes or ependymal cells generated by radial glia are multipotent stem cells. Luckily, this can now be directly assessed, as the reporter proteins encoded by the retroviral vectors will allow these cells to be sorted and collected. In particular, it will be of great interest to find out whether purified radial glial cells are multipotent stem cells or just committed glioblasts.

Why do radial glial cells transform into astrocytes despite the sustained activation of the Notch pathway? Nrg1/GGF2 helps to induce radial glial elongation and radial glial differentiation (Anton et al., 1997). The normal loss of radial glia at the end of neurogenesis might thus be triggered by the loss of Nrg1/GGF2, which is made at high levels by newly formed neurons but downregulated as they mature (Anton et al., 1997). Consistent with this possibility, when embryonic day 17 mouse neocortical neurons are transplanted into adult gray matter, nearby astrocytes elongate and take on both the morphology and antigenic markers of radial glial cells (Leavitt et al., 1999). Possibly, this morphological transformation is induced by Nrg1 acting together with Notch ligands expressed by the transplanted embryonic neurons. In any case, these observations suggest that radial glia and at least some astrocytes are interchangeable phenotypes, a finding supported by previous culture studies (Hunter and Hatten, 1995). These results provide hope that when quiescent neural stem cells within the adult brain can someday be stimulated to generate new neurons after brain injury, the new neurons might be able to trigger their own radial glial scaffold in order to migrate to where they are needed.

Does Notch Signaling Mediate the Developmental Switch between Neurogenesis and Gliogenesis?

Why does glial generation occur after neurogenesis? In the PNS, it has previously been proposed that gliogenesis follows neurogenesis because feedback signals made by neurons inhibit neurogenesis and stimulate gliogenesis. For instance, when purified NCSCs are cultured, they first generate neurons and later generate glia, possibly because GGF2 produced by the neurons acts on NCSCs to inhibit neurogenesis and stimulate gliogenesis (Shah et al., 1994). Morrison et al. (2000) found, however, that Notch activation of NCSCs promoted gliogenesis more rapidly and strongly than did GGF2. Thus, the Notch ligands expressed by newly formed neurons might feed back onto precursor cells to enhance gliogenesis, as Morrison et al. (2000) now propose. Whereas Notch signaling would be short range, GGF2 signaling might be longer range as Nrg1 exists in secreted as well as membrane-bound forms; Notch and GGF2 signaling might well be synergistic. Importantly, because the glia-inducing effect of Notch is dominant to the neuron-inducing effects of BMP2, glia could be generated by NCSCs in local environments

containing high levels of BMP2. In addition to such extracellular mechanisms that may enhance gliogenesis later in development, intrinsic mechanisms may operate as well. An interesting possibility would be that an intrinsic decrease in levels of numb, an intracellular protein that inhibits Notch signaling, in NCSCs over time could help to switch neurogenesis to gliogenesis.

What would be the virtue of having a long-range and a short-range signal cooperate in promoting gliogenesis? A possibility is that one signal might control the location of differentiation, and the other might control the cell fate generated. For instance, developing embryonic PNS neurons express Notch ligands on their axons as well as their cell bodies. As multipotent NCSCs migrate into prenatal peripheral nerves, those that happen to contact axons might be signaled by Notch activation to enter (or commit to entering) the Schwann cell lineage, whereas axonally released Nrg1/GGF2 might simultaneously promote their survival, proliferation, and further differentiation. Axonal Notch ligands might well have a second action on a later stage of the Schwann cell lineage to inhibit differentiation of immature Schwann cells into more mature, myelinating Schwann cells, delaying myelination until the appropriate postnatal time (Wang et al., 1998; our unpublished data). In this way, contact-mediated Notch signaling and soluble Nrg1 signaling might cooperate to match spatially and numerically the number of Schwann cells to the axons they will myelinate.

All in all, the new studies of Furukawa et al. (2000), Gaiano et al. (2000), and Morrison et al. (2000) provide an important and fascinating step forward in understanding how glial cells are generated. Although these studies raise as many questions as they answer, they provide important new clues into how neural stem cells may one day be manipulated to reconstruct a damaged nervous system.

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